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Nitrogen-containing heterocyclic compounds from the roots of *Callerya speciosa*

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Abstract:

Callerya speciosa (Champ.) Schot. [synonym: Millettia speciosa Champ.] belongs to the family Leguminosae, which grows in Southeast Asia, in the tropical and subtropical forests of Hainan Island, southern mainland China and in the north areas of Vietnam. C. speciosa have been used as a traditional medicine for the treatment of fever, cough, headache, backache, rheumatism, chronic bronchitis, and nephritis. The presence of phenolics, phenolic glycosides, pterocarpans, flavonoids, isoflavonoids, sterols, and chromones in this species have been reported. In this study, the four nitrogen-containing heterocyclic compounds uridine (1), 2-(β-D-glucopyranosyl)-3-isoxazolin-5-one (2), adenosine (3), and hypaphorine (4) were isolated from the n-butanol extract of the roots of Callerya speciosa collected in Vietnam. The compounds were isolated and purified by silica gel, Sephadex LH-20 and RP-18 column chromatography. As a result, their structures were characterized on the basis of extensive NMR, mass spectroscopic analyses, and comparison with reported values. This is the first report on the isolation of compounds 1–4 from Callerya speciosa.

Keywords: ***Callerya speciosa*****, Leguminosae, nitrogen-containing heterocyclic compounds.**

Classification number: **3.3**

1. Introduction

Callerya speciosa (Champ.) Schot. [synonym: *Millettia speciosa* Champ.] is a valuable medicinal plant belonging to the family Leguminosae, which is widely distributed in Southeast Asia in the tropical and subtropical forests of Hainan Island and southern mainland China [1]. *C. speciosa* is also found in the north areas of Vietnam such as Tuyen Quang, Bac Kan, Bac Giang, and Phu Tho. In recent years, *C. speciosa* has been planted more frequently in areas of Vietnam as its roots have been used as a traditional medicine for the treatment of fever, cough, headache, backache, rheumatism, chronic bronchitis, and nephritis [2]. Previous phytochemical studies on this species revealed the presence of phenolics, phenolic glycosides, pterocarpans, flavonoids, isoflavonoids, sterols, and chromones [3-9]. In a previous work, we reported the isolation and structural characterization of a new oleanane triterpenoid along with three known compounds from the ethyl acetate extract of the *C. speciosa* roots [10]. In a further investigation of chemical constituents from the n-butanol extract of the roots of this species, we isolated four nitrogen-containing compounds including uridine (**1**), 2-(*β*-D-glucopyranosyl)-3-isoxazolin-5-one (**2**), adenosine (**3**), and hypaphorine (**4**), and their structures were fully characterized.

2. Materials and methods

2.1. General experiment procedure

1D- and 2D-NMR spectra were acquired on a Bruker Avance 500 Ultrashield NMR Spectrometer. ESI-MS was measured on an Agilent LC-MSD-Trap SL. Thin layer chromatography was carried on silica gel 60 F254 (0.25 mm, Merck) and reversed phase RP18 F254S (0.25 mm, Merck) plates. Column chromatography was performed using silica gel 60 (230-400 mesh, Merck), YMC RP-18 resins (30-50 μm, Fuji Silysia Chemical Ltd), and Sephadex LH-20 gel (Amersham Pharmacia Biotech).

2.2. Plant material

The plant material was collected in the Tan Yen district, Bac Giang province, in March of 2018. The sample identification was done by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST), and a voucher specimen was preserved in the Laboratory of Natural Products Research, Institute of Chemistry, VAST.

2.3. Extraction and isolation

The powdered roots of *C. speciosa* (870 g) were extracted with 95% methanol three times at room

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temperature. The extracts were filtered, combined, and concentrated under reduced pressure. The obtained residue was successively dissolved with water and re-extracted in turn with ethyl acetate and n-butanol. The organic solvents were concentrated to give 4.6 g and 9.7 g of the corresponding extracts.

The n-butanol extract was fractionated by silica gel column chromatography eluted with CH_2Cl_2:MeOH:H_2O (4:1:0.1 - 1.5:1:0.2, v/v) to give 7 fractions. Fraction 2 was re-purified by silica gel column (CH_2Cl_2:MeOH:H_2O = 4:0.8:0.1, v/v), then Sephadex LH-20 column (MeOH) to afford compound **1** (4 mg). Fraction 4 was re-purified on a silica gel column (CH_2Cl_2:MeOH:H_2O = 3:1:0.1, v/v), then Sephadex LH-20 column (MeOH) to give compounds **2** (3 mg) and **3** (4 mg). Fraction 6 was chromatographed on a reversed phase silica gel (RP-18) column (MeOH:H_2O = 1:1), then silica gel column (CH_2Cl_2:MeOH:H_2O = 2.5:1:0.1, v/v) to yield compound **4** (7 mg).

Uridine (1):

Yellowish solid. ESI-MS: *m/z*267.2 $[M+Na]^+$. ^{1}H-NMR (500 MHz, CD_3OD): δ_H 8.02 (1H, d, *J*=7.0 Hz, H-6), 5.92 (1H, d, *J*=4.0 Hz, H-1′), 5.71 (1H, d, *J*=7.0 Hz, H-5), 4.21-4.19 (1H, m, H-2′), 4.18-4.16 (1H, m, H-3′), 4.03-4.02 (1H, m, H-4′), 3.86 (1H, dd, *J*=10.0, 2.0 Hz, H-5a′), 3.75 (1H, dd, *J*=10.0, 2.0 Hz, H-5b′). ^{13}C-NMR (125 MHz, CD_3OD): δ_C166.19 (C-4), 152.47 (C-2), 142.73 (C-6), 102.66 (C-5), 90.75 (C-1′), 86.37 (C-4′), 75.72 (C-2′), 71.31 (C-3′), 62.28 (C-5′).

2-(*β*-D-glucopyranosyl)-3-isoxazolin-5-one (2):

Colourless solid. ESI-MS: *m/z*248.1 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD): δ_H 8.44 (1H, d, *J*=3.0 Hz, H-3), 5.33 (1H, d, *J*=3.0 Hz, H-4), 4.92 (1H, d, *J*=7.5 Hz, H-1′), 3.87-3.85 (1H, m, H- H-6a′), 3.69-3.67 (1H, m, H-6b′), 3.61-3.60 (1H, m, H-2′), 3.46 (1H, t, *J*=7.5 Hz, H-3′), 3.41-3.40 (1H, m, H-5′), 3.36 (1H, d, *J*=7.5 Hz, H-4′). ^{13}C-NMR (125 MHz, CD_3OD): δ_C 173.96 (C-5), 154.73 (C-3), 90.86 (C-4), 90.43 (C-1′), 80.39 (C-5′), 78.67 (C-3′), 73.85 (C-2′), 70.93 (C-4′), 62.46 (C-6′).

Adenosine (3):

White solid. ESI-MS: *m/z* 268.1 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD): δ_H 8.32 (1H, s, H-8), 8.20 (1H, s, H-2), 5.99 (1H, d, *J*=6.5 Hz, H-1′), 4.76 (1H, dd, *J*=6.0, 5.5 Hz, H-2′), 4.35 (1H, dd, *J*=5.0, 3.0 Hz, H-3′), 4.19 (1H, dd, *J*=3.5, 3.0 Hz, H-4′), 3.91 (1H, dd, *J*=12.5, 3.0 Hz, H-5a′), 3.77 (1H, dd, *J*=12.5, 3.0 Hz, H-5b′). ^{13}C-NMR (125 MHz, CD_3OD): δ_C 157.41 (C-6), 153.52 (C-2), 150.02 (C-4), 142.01 (C-8), 121.24 (C-5), 91.26 (C-1′), 88.17 (C-4′), 75.48 (C-2′), 72.65 (C-3′), 63.47 (C-5′).

Hypaphorine (4):

Colourless solid. ESI-MS: *m/z* 247.3 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD): δ_H 7.67 (1H, dd, *J*=6.5, 1.0 Hz, H-5), 7.39 (1H, dd, *J*=6.5, 1.0 Hz, H-8), 7.23 (1H, s, H-2), 7.15 (1H, dt, *J*=6.0, 1.0 Hz, H-7), 7.09 (1H, dt, *J*=6.0, 1.0 Hz, H-6), 3.91 (1H, t, *J*=6.0 Hz, H-11), 3.44 (2H, d, *J*=6.0 Hz, H-10), 3.29 [9H, s, -$N^+(CH_3)_3$]. ^{13}C-NMR (125 MHz, CD_3OD): δ_C 171.34 (C-12), 137.98 (C-9), 128.37 (C-4), 125.16 (C-2), 122.63 (C-7), 120.07 (C-6), 119.22 (C-5), 112.55 (C-8), 109.21 (C-3), 80.58 (C-11), 52.73, 52.71, 52.69 [-$N^+(CH_3)_3$], 24.62 (C-10).

3. Results and discussion

Compound **1** was obtained as yellowish solid. The ^{13}C-NMR and HSQC spectra of compound **1** revealed resonances for nine carbons of which four carbons [δ_C 166.19 (C-4), 152.47 (C-2), 142.73 (C-6), 102.66 (C-5)] were assigned to a uracil and five carbons [δ_C 90.75 (C-1′), 86.37 (C-4′), 75.72 (C-2′), 71.31 (C-3′), 62.28 (C-5′)] to a ribofuranosyl unit. The ^{1}H-NMR spectrum showed signals of a pair of doublets at δ_H 8.02 (1H, d, *J*=7.0 Hz, H-6) and 5.71 (1H, d, *J*=7.0 Hz, H-5); a ribofuranose with an anomeric proton signal at δ_H 5.92 (1H, d, *J*=4.0 Hz, H-1′); and other sugar protons in regions δ_H 4.21-3.75 ppm. The HMBC spectrum showed correlations from H-1′ (δ_H 5.92) to C-2 (δ_C 152.47) and C-6 (δ_C 142.73) indicating uracil linked to a ribofuranose via a β-N1-glycosidic bond. The molecular formula of **1** was deduced to be $C_9H_{12}N_2O_6$ based on NMR data and an ESI-MS pseudo-molecular ion peak at *m*/z 267.2 $[M+Na]^+$. The ^{1}H- and ^{13}C-NMR data from compound **1** were consistent with uridine in literature [11]. Therefore, compound **1** was elucidated to be uridine.

Compound **2** was isolated as colourless solid. The ^{1}H-NMR spectrum of compound **2** shows typical signals of isoxazolin-5-one with two doublets of H-3 and H-4 at δ_H 8.44 and 5.33, respectively ($^3J_{3,4}$=3.0 Hz). In addition, the signals of a *β*-D-glucopyranosyl unit with an anomeric proton signal at δ_H 4.92 (1H, d, *J*=7.5 Hz, H-1′) and complex proton signals in the region of δ_H 3.87-3.36 were also observed. Corresponding to the ^{1}H-NMR spectrum, the ^{13}C-NMR of **2** showed one carbonyl (δ_C 173.96), two methine carbons (δ_C 154.73,90.86), and a glucose unit (δ_C 90.43, 80.39, 78.67, 73.85, 70.93, and 62.46). The Heteronuclear Multiple Bond correlation (HMBC) correlation between H-1′ (δ_H 4.92) and C-3 (δ_C 154.73) were detected. A molecular formula of $C_9H_{13}NO_7$ was determined for compound **2** on the basis of an ion peak $[M + H]^+$ at *m/z* 248.1 in ESI-MS and NMR data.

Based on this evidence and comparison with the reported data [12], the structure of **2** was determined to be 2-(*β*-D-glucopyranosyl)-3-isoxazolin-5-one.

Compound **3** was isolated as a white solid. The ^{1}H- and ^{13}C-NMR spectra of **3** showed the presence of an adenine unit with three quaternary carbons at δ_C 157.41 (C-6), 150.02 (C-4), and 121.24 (C-5) and two methane groups [δ_H 8.32 (1H, s, H-8), δ_C 142.01 (C-8); δ_H 8.20 (1H, s, H-2), δ_C 153.52 (C-2)]. In addition, the signals of a ribofuranosyl moiety with four methane groups at δ_H5.99 (1H, d, *J*=6.5 Hz, H-1′), δ_C 91.26 (C-1′); δ_H 4.76 (1H, dd, *J*=6.0, 5.5 Hz, H-2′), δ_C 75.48 (C-2′); δ_H 4.35 (1H, dd, *J*=5.0, 3.0 Hz, H-3′), δ_C 72.65 (C-3′); δ_H 4.19 (1H, dd, *J*=3.5, 3.0 Hz, H-4′), δ_C 88.17 (C-4′)], and one methylene group at δ_H 3.91 (1H, dd, *J*=12.5, 3.0 Hz, H-5a′), 3.77 (1H, dd, *J*=12.5, 3.0 Hz, H-5b′), δ_C 63.47 (C-5′)] were observed. The HMBC correlations from H-1′ (δ_H 5.99) to C-4 (δ_C 150.02), and C-8 (δ_C 142.01) suggested that an adenine attached to a ribose sugar molecule via a *β*-N_9-glycosidic bond. Its molecular formula was established as $C_{10}H_{13}N_5O_4$ by a combination of NMR data and an ESI-MS pseudo-molecular ion peak at *m/z* 268.1 $[M+H]^+$. From the above spectral data, the structure of **3** was determined to be adenosine. The ^{1}H- and ^{13}C-NMR data (in CD_3OD) of **3** resemble those of adenosine in literature [13].

Compound **4** was obtained as a colourless solid. The ^{1}H-NMR spectrum of **4** exhibited signals characteristic of 3-substituted indole skeleton with four aromatic vicinal protons of a disubstituted benzene ring at δ_H 7.67 (1H, dd, *J*=6.5, 1.0 Hz, H-5), 7.39 (1H, dd, *J*=6.5, 1.0 Hz, H-8), 7.15 (1H, dt, *J*=6.0, 1.0 Hz, H-7), 7.09 (1H, dt, *J*=6.0, 1.0 Hz, H-6); and one singlet at δ_H 7.23 (1H, s, H-2). The signals of the side chain including three protons at δ_H 3.91 (1H, t, *J*=6.0 Hz, H-11), 3.44 (2H, d, *J*=6.0 Hz, H-10); and three methyl groups at δ_H 3.29 (9H, s) were also observed. The ^{13}C-NMR spectrum of **4** showed one carbonyl group (δ_C 171.34), five sp^2 methines (δ_C 125.16, 122.63, 120.07, 119.22. 112.55), three sp^2 quaternary carbons (δ_C 137.98, 128.37, 109.21), one nitrogen-bearing sp^3 methine (δ_C 80.53), one sp^3 methylene (δ_C 24.62), and three methyls (δ_C 52.73, 52.71, 52.69). The HMBC correlations between the methyl protons at δ_H 3.29 with these methyl carbons at δ_C 52.73, 52.71, 52.69, and methine carbon at δ_C 80.53 suggested these three methyls connected together with one nitrogen and -$N^+(CH_3)_3$ group linked with methine carbon C-11. In additional, the HMBC spectrum exhibited correlations from protons H-10 (δ_H 3.44) to carbons C-2 (δ_C 125.16), C-3 (δ_C 109.21), C-4 (δ_C 128.37) and C-12 (δ_C 171.340; from H-11 (δ_H 3.91) to C-3 (δ_C 3.91), and C-12 (δ_C 171.34). The molecular formula of **4** was determined to be $C_{14}H_{18}N_2O_2$ from ESI-MS molecular ion peak at *m*/z 247.3 $[M+H]^+$ and NMR data. Based on this evidence and comparison with the reported values in literature [14], compound **4** was determined to be hypaphorine (Fig. 1).

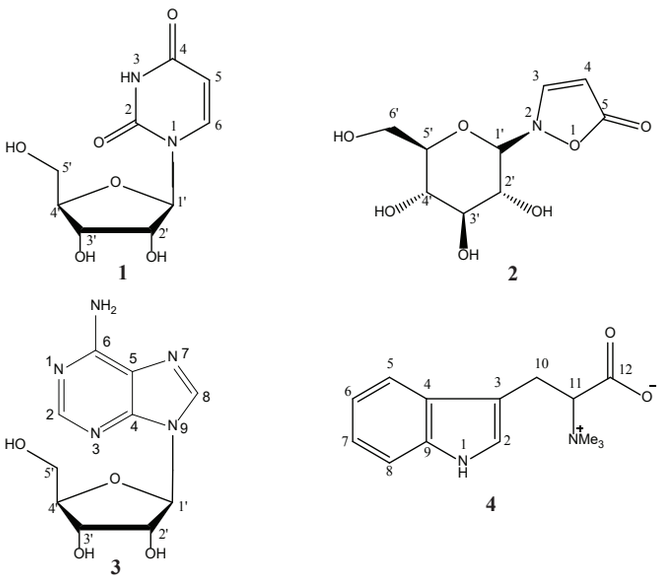

Fig. 1. Structures of compounds 1-4.

4. Conclusions

Column chromatography of the n-butanol extract of *Callerya speciosa* roots resulted in the isolation of four nitrogen-containing heterocyclic compounds: uridine (**1**), 2-(*β*-D-glucopyranosyl)-3-isoxazolin-5-one (**2**), adenosine (**3**), and hypaphorine (**4**). The chemical structures of compounds **1-4** were established by MS and NMR. All these compounds were isolated from this species for the first time.

CRediT author statements

Dao Duc Thien: Methodology, Data curation, Writing original draft preparation; Le Quoc Thang: Writing original draft preparation; Nguyen Thanh Tam: Writing - Reviewing and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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